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Title: *BDNF* SNPs are implicated in comorbid alcohol dependence in schizophrenia but not in alcohol dependent patients without schizophrenia

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Running title: *BDNF* association with comorbid alcohol dependence

## Abstract

**Aims:** The functional *BDNF* single nucleotide polymorphism (SNP) rs6265 has been associated with many disorders including schizophrenia and alcohol dependence. However, studies have been inconsistent, reporting both positive and negative associations. Comorbid alcohol dependence has a high prevalence in schizophrenia so we investigated the role of rs6265 in alcohol dependence in Australian populations of schizophrenia and alcohol dependent patients.

**Methods:** Two *BDNF* SNPs rs6265 and a nearby SNP rs7103411 were genotyped in a total of 848 individuals. These included a schizophrenia group (n = 157) and a second schizophrenia replication group (n = 235), an alcohol dependent group (n = 231) that had no schizophrenia diagnosis and a group of healthy controls (n = 225).

**Results:** Allelic association between rs7103411 and comorbid alcohol dependence was identified ( $P = 0.044$ ) in the primary schizophrenia sample. In the replication study, we were able to detect allelic associations between both *BDNF* SNPs and comorbid alcohol dependence (rs6265,  $P = 0.006$ ; rs7103411,  $P = 0.014$ ). Moreover, we detected association between both SNPs and risk-taking behaviour after drinking (rs6265,  $P = 0.005$ ; rs7103411,  $P = 0.009$ ) and we detected strong association between both SNPs and alcohol dependence in males (rs6265,  $P = 0.009$ ; rs7103411,  $P = 0.013$ ) while females showed association with multiple behavioural measures reflecting repetitive alcohol consumption. Haplotype analysis revealed the rs6265-rs7103411 A/C haplotype is associated with comorbid alcohol dependence ( $P = 0.002$ ). When these SNPs were tested in the non-schizophrenia alcohol dependent group we were unable to detect association.

**Conclusion:** We conclude that these *BDNF* SNPs play a role in development of comorbid alcohol dependence in schizophrenia while our data does not indicate that they play a role in alcohol dependent patients who do not have schizophrenia.

**Keywords:** comorbid alcohol dependence, schizophrenia, *BDNF*, polymorphism

## 1. Introduction

Schizophrenia is a highly heritable and complex disorder affecting 1% of the global population. It is a syndrome consisting of a varying number of psychological phenotypes depending on the interaction between the genetic background and environmental exposure of those affected. One of the most common comorbid phenotypes found in schizophrenia is alcohol dependence. Previous studies reported a prevalence of up to 55% alcohol dependence in schizophrenia cases (Koskinen et al., 2009).

Many genes with a direct neurobiological role have been studied in relation to schizophrenia. Brain-derived neurotrophic factor (encoded by the *BDNF* gene) regulates proliferation and survival of nerve cells in the central and peripheral nervous systems (Hartmann et al., 2001). Additionally, *BDNF* influences the activity of dopaminergic systems (Ghosh et al., 1994; Guillin et al., 2001). Changes in *BDNF* protein, mRNA or receptor levels in the hippocampus and cortical regions of the brain have been reported in schizophrenia patients (Durany et al., 2001; Takahashi et al., 2000; Weickert et al., 2003), implying that *BDNF* alteration plays a role in schizophrenia pathogenesis. Hence *BDNF* is a suitable candidate gene for study in schizophrenia.

A functional *BDNF* single nucleotide polymorphism (SNP) rs6265 has been studied extensively because it is within the coding sequence and results in a Val66Met amino acid sequence change. The Met allele is believed to alter intracellular pro-BDNF (immature BDNF) trafficking and packaging, therefore altering regulated secretion of mature BDNF (Hariri et al., 2003). Several studies have reported association between rs6265 and schizophrenia (Chao et al., 2008; Zakharyan et al., 2011), positive symptoms in

schizophrenia (Zhai et al., 2013), and drug response in schizophrenia (Zai et al., 2012b; Zhang et al., 2013). However, several studies failed to detect associations between rs6265 and schizophrenia (Kanazawa et al., 2007; Li et al., 2013; Suchanek et al., 2013; Watanabe et al., 2006) and onset age of schizophrenia (Decoster et al., 2011). Although the biological role of *BDNF* strongly suggests involvement in schizophrenia, the inconsistent findings between rs6265 and schizophrenia raises the question of whether rs6265 is involved in a specific endophenotype rather than schizophrenia in general. The rs6265 *BDNF* SNP has also shown association with a number of other psychiatric phenotypes. A schizophrenia family study found association with rs6265, particularly with cognitive deficits (Rosa et al., 2006) but a previous family study failed to find association (Egan et al., 2003). Other studies found association between rs6265 and alcohol abuse and related phenotypes (Benzerouk et al., 2013; Colzato et al., 2011; Shin et al., 2010; Wojnar et al., 2009). These studies are also consistent with human and animal studies showing that low *BDNF* levels are found in alcohol dependence and *BDNF* down-regulates alcohol induced dopaminergic elevation (Bosse and Mathews, 2011; Matsushita et al., 2004; Wojnar et al., 2009).

One study showed that a haplotype involving the *BDNF* SNPs rs7103411 and rs4923463 was associated with comorbid alcohol dependence in bipolar disorder (Neves et al., 2011). The rs4923463 SNP is in the 3'-flanking region of *BDNF* and rs7103411 is in intron 7 but the functional impact of these SNPs on *BDNF* is unknown.

A number of studies identified the impact of gender on the effect of *BDNF* polymorphisms on various psychiatric phenotypes (Manning and van den Buuse, 2013; Nishichi et al., 2013; Suchanek et al., 2013). This highlights the importance of gender influence on the function of *BDNF* which could explain the inconsistencies between association studies involving *BDNF*.

The objective of this study was to investigate the role of the *BDNF* SNPs rs6265 and rs7103411 in comorbid alcohol dependence in Australian populations of schizophrenia patients and in alcohol dependent patients without schizophrenia. Additionally, the effects of gender on the role of the *BDNF* SNPs in alcohol dependence in both groups were investigated.

## **2. Materials and Methods**

### *2.1 Subjects*

Two schizophrenia patient groups, one alcohol dependent group and a healthy control group were recruited. The second schizophrenia group served as a replication group.

#### *2.1.1 Schizophrenia Group 1*

Schizophrenia group one included 157 schizophrenia patients of European descent who were aged between 18 and 65 years. Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) diagnosis of schizophrenia was confirmed by at least two independent psychiatrists. Patients were also screened for alcohol use disorder (AUD) using the Alcohol Use Disorder Identification Test (AUDIT). Patients scoring at least 12 in AUDIT are considered alcohol dependent. AUDIT scores below 12 are considered not alcohol dependent. These patients had never been diagnosed with other psychiatric disorders, including schizoaffective disorder, major depressive episode with psychotic features, or bipolar disorder. No patients were treated with antidepressants, anxiolytic agents, or mood-stabilizing psychotropic medications and all were maintained on a constant dose of antipsychotic medication for a minimum of three weeks.

There were 23 females and 134 males in the group diagnosed with schizophrenia with a mean age of 36.2 years (s.d.  $\pm$  12.1 years). The mean age of patients at first diagnosis (onset of psychotic symptoms) was 23.2 years (s.d.  $\pm$  7.3 years). They were being treated at a large



teaching hospital, an inpatient treatment facility or a community mental health centre. The clinical sample was composed of 65 inpatients and 92 outpatients.

This clinical sample was a group who had lived with schizophrenia for an average of 13 years since diagnosis and continued to experience positive and negative symptoms despite treatment with antipsychotic medication. Mean length of illness was 13.39 years (s.d  $\pm$  10.91). In addition, the schizophrenia group contained a high proportion of individuals with a relatively severe history and/or a familial risk for psychosis.

### *2.1.2 The Replication Schizophrenia Group*

The second schizophrenia patient group was obtained from the Australian Schizophrenia Research Bank (ASRB: [www.schizophreniaresearch.org.au](http://www.schizophreniaresearch.org.au)) and included 235 schizophrenia patients of European descent with a mean age of 43.9 years (s.d  $\pm$  10.7 years). There were 70 females and 165 males with a confirmed diagnosis of schizophrenia according to DSM-IV/ICD-10 diagnostic criteria with a first diagnosis mean age of 39.4 years (s.d  $\pm$  10.7 years). All participants underwent a clinical and neuropsychological assessment and provided a blood sample at the time of assessment. The participants were assessed using a clinical assessment battery that consists of the Diagnostic Interview for Psychosis (DIP) (Castle et al., 2006) to collect socio-demographic, family and medical history data, and to screen for or confirm diagnosis.

The patients were recruited from several sources across five Australian States and Territories (New South Wales, Australian Capital Territory, Queensland, Western Australia and Victoria) using media advertisements, inpatient, outpatient and community mental health

service providers, non-government organisation, rehabilitation services. To determine European descent, the country of origin for the participant, their parents and all grandparents, were recorded. Europe was defined as continental Europe and the British Isles (including Turkey but excluding Middle Eastern Europe). Australians of Aboriginal/Torres Strait Island descent were excluded.

### *2.1.3 Alcohol Dependent Group*

A total of 231 unrelated alcohol dependent subjects of European descent (74 female and 157 male) were recruited from large public hospitals in Brisbane, Australia. The mean age of the group was 40.74 years (s.d.  $\pm$  10.3 years). All subjects were assessed against a checklist of specific criteria by a clinical psychologist experienced in drug and alcohol dependence and met DSM-IV criteria of alcohol dependence disorder. All were inpatients and represented a spectrum of severity with a significant proportion ( $n = 65$ ) of these patients being diagnosed with two or more alcohol related medical conditions such as pancreatitis, cirrhosis, hepatitis or peripheral neuropathy. Alcohol dependent patients were excluded from the study if they had dementia, delirium, psychosis, or any other condition that would affect their ability to provide informed consent. A large proportion (81%) of the alcohol dependent cohort was also smokers.

### *2.1.4 Control Group*

The control group was also obtained from Australian Schizophrenia Research Bank. The controls consisted of 121 females and 104 males of European descent, with a mean age of 45.00 years (s.d.  $\pm$  13.2 years). Healthy controls were screened for a family history of, or

treatment for, a psychiatric illness at the time of registration. The controls underwent a clinical and neuropsychological assessment and provided a blood sample at the time of assessment. The controls were assessed using a clinical assessment battery that consists of the Diagnostic Interview for Psychosis (DIP) (Castle et al., 2006) to collect socio-demographic, family and medical history data.

### *2.1.5 Ethics Approval*

Ethics approval for the project was obtained from the Human Research Ethics Committee of the Queensland University of Technology.

## *2.2 SNP Genotyping*

Samples were genotyped for the *BDNF* SNPs rs6265 and rs710341 using a homogeneous MassEXTEND (hME) Sequenom assay performed by the Australian Genome Research Facility.

## *2.3 Statistical Analysis*

Association between SNPs and schizophrenia and/or alcohol dependence were determined using  $\chi^2$  tests by comparing frequencies between schizophrenia patients and the control group, or schizophrenia patients with and without alcohol dependence. Clinical measures were analysed in SPSS using ANOVA (analysis of variance). Patients in all groups were analysed according to gender and power calculations revealed that a sample size of 244 case and 244 non-case alleles is required to achieve 80% power at an odds ratio of 1.8 for both

SNPs. In schizophrenia group one, females had 2 case and 38 non-case alleles and males had 82 case and 158 non-case alleles; in the schizophrenia replication group, females had 124 case and 238 non-case alleles and males had 296 case and 202 non-case alleles; in the alcohol dependent group, females had 124 case and 238 non-case alleles and males had 82 case and 158 non-case alleles. Statistical tests were performed using Compare2 (ver. 2.75) (Abramson, 2004) and Statistical Package for the Social Sciences (SPSS) 21. Hardy-Weinberg equilibrium was computed using Utility Programs for Analysis of Genetic Linkage (Ott, 1988) and haplotypes were analysed using JLIN (Carter et al., 2006). Correction for multiple testing for  $\chi^2$  tests was conducted using the Benjamini-Hochberg method (Benjamini and Hochberg, 1995).

### 3. Results

#### 3.1 Schizophrenia Group One

Genotypes for rs6265 and rs7103411 were determined in 42 schizophrenia cases with comorbid alcohol use disorder and 99 schizophrenia cases without comorbid alcohol use disorder. The *BDNF* SNPs were first tested for association with schizophrenia by comparing genotype frequencies between schizophrenia group one and the control group. Schizophrenia was not significantly associated with rs6265 ( $P = 0.857$ ) nor rs7103411 ( $P = 0.579$ ) at the genotype level. All genotype frequencies were found to be in Hardy-Weinberg equilibrium for both the schizophrenia and control populations.

We then tested both SNPs for association with alcohol-related phenotypes within schizophrenia. Patients in schizophrenia group one were screened for alcohol use disorders using the Alcohol Use Disorders Identification Test (AUDIT). A cut-off score of 12 was used instead of the recommended score, 8 to achieve higher level of specificity, therefore reducing false positives (Babor et al., 2001). Patients scoring at least 12 are considered alcohol dependent while scores below 12 are considered not alcohol dependent. We also further tested the SNPs against alcohol-related behavioural measures to identify if the SNPs were associated with specific alcohol-related behavioural variability. The alcohol-related behaviours were measured using the Drinking Expectancy Questionnaire (DEQ) and Drinking Refusal Self Efficacy Questionnaire (DRSEQ), which measure behavioural change upon alcohol consumption and ability to refrain from alcohol use (Li and Dingle, 2012; Oei et al., 2005) respectively. Lastly, haplotype analysis was performed to investigate association between haplotypes and comorbid alcohol dependence.

Genotypes for rs6265 were marginally associated ( $P = 0.046$ ) with comorbid alcohol dependence after trend analysis (Table 1) but rs6265 was not associated at the allele level (Table 2). The rs7103411 SNP was associated at genotype level after trend analysis ( $P = 0.014$ ) and at the allele level ( $P = 0.044$ ), with TT being the low-risk genotype (Table 1). ANOVA did not detect any significant differences between the SNP genotypes and DEQ scores, DRSEQ scores and drinking quantities over time. Haplotype analysis revealed strong linkage disequilibrium (LD) ( $D' = 1$ ) between rs6265 and rs7103411 but no significant haplotype association with comorbid alcohol dependence was detected ( $P = 0.195$ ).

When the schizophrenia patients were grouped according to gender, neither rs6265 nor rs7103411 were associated at the allele or genotype level. However, in the male schizophrenia patients, trend analysis revealed association between comorbid alcohol dependence and both rs6265 ( $P = 0.031$ ), and rs7103411 ( $P = 0.012$ ) genotypes. At the allele level, only rs7103411 was associated with comorbid alcohol dependence ( $P = 0.036$ ). ANOVA did not detect any significant differences between both SNP genotypes and DEQ scores, DRSEQ scores and drinking quantities over time in both male and female patients.

### 3.2 *The Replication Schizophrenia Group*

Genotypes for rs6265 and rs7103411 were determined for the second set of schizophrenia cases and controls. In total, 72 schizophrenia cases with comorbid alcohol dependence and 162 schizophrenia cases without comorbid alcohol dependence were genotyped.

No association with schizophrenia was detected for either rs6265 ( $P = 0.731$ ) or rs7103411 ( $P = 0.910$ ) at the genotype level.

When comorbid alcohol dependence was examined among the cases associations with both rs6265 ( $P = 0.013$ ) and rs7103411 ( $P = 0.017$ ) were detected at the genotype level (Table 3). Mantel-Haenszel trend test also revealed significant comorbid alcohol dependence association for both rs6265 ( $P = 0.003$ ) and rs7103411 ( $P = 0.006$ ) genotypes using a partial dominance model (Table 3). The rs6265 odds ratio suggests a trend from the low-risk GG genotype, followed by AG and AA being the high-risk genotype (Table 3). As for rs7103411, TT is the lowest risk genotype, followed by CT and CC (Table 3). In agreement with the partial dominance model, allele association also revealed significant association between the high-risk alleles (rs6265 A allele and rs7103411 C allele) and comorbid alcohol dependence (Table 4).

ANOVA was performed to identify SNP associations with alcohol-related behavioural measures. These measures were scored based on the scale of clinical assessment battery from the Diagnostic Interview for Psychosis (DIP) under the ‘alcohol use’ domain. The measures include drinks consumed per session, risk taking behaviour, alcohol craving, inability to stop drinking, alcohol tolerance, withdrawal, social problems related to alcohol, attempts to reduce alcohol consumption, guilt from alcohol consumption and need for morning alcohol consumption. Risk taking behaviour (use of alcohol in situations increasing risk of injury to self and others) was found associated with both rs6265 ( $P = 0.005$ ,  $F = 5.577$ ) and rs7103411 ( $P = 0.009$ ,  $F = 4.860$ ) in schizophrenia (Figure 1). High ‘risk taking behaviour’ scores were associated with the rs6265 AA genotype and the rs7103411 CC genotype. Haplotype analysis revealed an overall haplotype association with comorbid alcohol dependence ( $P =$

0.008), with the high-risk AC haplotype strongly associated with alcohol dependence ( $P = 0.002$ ). No other alcohol-related behavioural measures were found associated with rs6265 or rs7103411.

When analysed by gender, neither rs6265 ( $P = 0.077$ ) nor rs7103411 ( $P = 0.059$ ) were associated with comorbid alcohol dependence in females but they did show association with alcohol-related behavioural measures; “need for morning alcohol consumption” ( $P = 0.004$ ,  $F = 6.121$ ), “attempts to reduce alcohol consumption” ( $P = 0.028$ ,  $F = 3.928$ ), “alcohol tolerance” ( $P = 0.001$ ,  $F = 7.738$ ), “withdrawal” ( $P = 0.003$ ,  $F = 6.862$ ), and “social problems related to alcohol” ( $P = 0.001$ ,  $F = 7.639$ ) for the AA genotype of rs6265. These measures also survived post-hoc Tukey test for AA compared with AG and GG genotypes, except “attempts to reduce alcohol consumption” achieved near significance between AA and AG genotypes (Tukey’s  $P = 0.052$ ). Similarly, the CC genotype of rs7103411 was associated with the same measures of “need for morning alcohol consumption” ( $P = 0.012$ ,  $F = 4.853$ ), “attempts to reduce alcohol consumption” ( $P = 0.028$ ,  $F = 3.928$ ), “alcohol tolerance” ( $P = 0.001$ ,  $F = 7.738$ ), “withdrawal” ( $P = 0.003$ ,  $F = 6.862$ ), and “social problems related to alcohol” ( $P = 0.001$ ,  $F = 7.639$ ) and survived post-hoc tests except for “attempts to reduce alcohol consumption” (Tukey’s  $P = 0.052$ ) between CC and CT genotypes.

In male schizophrenia patients with comorbid alcohol dependence both rs6265 ( $P = 0.009$ ) and rs7103411 ( $P = 0.013$ ) genotypes and alleles, rs6265 ( $P = 0.016$ ) and rs7103411 ( $P = 0.028$ ) were associated. SNPs rs6265 and rs7103411 both survived corrections and there were significant differences between AA and AG (Tukey’s  $P = 0.024$ ), and between CC and CT (Tukey’s  $P = 0.036$ ) genotypes.



### 3.3 Alcohol Dependent Group

Genotypes for rs6265 and rs7103411 were determined for the alcohol dependent group in 210 cases and 220 controls. Neither SNP was associated with alcohol dependence at the genotype level and only rs6265 alleles showed marginal association ( $P = 0.046$ ), although this did not survive correction for multiple testing. Similarly, haplotypes were not associated ( $P = 0.208$ ) with alcohol dependence and no association was seen after analysing by gender.

## 4. Discussion

In this study, *BDNF* SNPs rs6265 and rs7103411 were not associated with schizophrenia in two schizophrenia sample groups. However, the SNPs were weakly associated with comorbid alcohol dependence in our first schizophrenia group and much stronger association was found with comorbid alcohol dependence for both rs6265 and rs7103411 when a larger replication schizophrenia group were tested. The differences in results between the original and the replication schizophrenia groups may be due to the smaller number of cases in the original group.

To our knowledge, no studies investigating associations of *BDNF* SNPs rs6265 and rs7103411 with comorbid alcohol dependence in schizophrenia have been published. Consistent with several previous studies (Decoster et al., 2011; Kanazawa et al., 2007; Li et al., 2013; Suchanek et al., 2013; Watanabe et al., 2006), we report no association between rs6265 and schizophrenia. Previous association studies involving rs6265 and alcohol

dependence have had inconsistent findings with some detecting association (Benzerouk et al., 2013; Colzato et al., 2011; Shin et al., 2010; Wojnar et al., 2009) while others failed to detect association (Nedic et al., 2013). Our study approached significance but we were not able to detect association between the two *BDNF* SNPs and alcohol dependence.

Interestingly, association was found with both rs6265 and rs7103411 in schizophrenia patients with comorbid alcohol dependence but there was no association in alcohol dependent individuals without schizophrenia. These results suggest a possible role of both *BDNF* SNPs in the aetiology of comorbid alcohol dependence phenotype in schizophrenia. Analysis of the *BDNF* SNPs and alcohol-related behaviour in the replication schizophrenia group revealed that both rs6265 and rs7103411 are associated with risk of self-injuries and injuries to others, which also has been reported in similar studies (Paska et al., 2013; Sarchiapone et al., 2008; Zai et al., 2012a). When schizophrenia patients were analysed by gender, both *BDNF* SNPs were associated with comorbid alcohol dependence in females but not males. Analysis of alcohol-related behaviour measures revealed that both *BDNF* SNPs were associated with a number of alcohol-related behaviour measures based on gender. In males, they were associated with risk taking behaviour, while females showed association with behaviours reflecting repetitive alcohol consumption. Our results are consistent with previous studies which reported *BDNF* variants induced gender-specific effects leading to gender-specific phenotypic outcomes (Manning and van den Buuse, 2013; Nishichi et al., 2013; Suchanek et al., 2013). One similar study however, did not find any gender-specific association in alcohol dependent subjects between *BDNF* and alcohol-related phenotypes including depression, aggression, suicidal behaviour, delirium and withdrawal (Nedic et al., 2013). These results strongly suggest that *BDNF* elicits different behavioural effects and plays a different aetiological role based on gender. In line with a mouse study, males with reduced *BDNF*

levels treated with amphetamine elicited reduced prepulse inhibition (a signal involved in the pathways for psychosis) but not in females (Manning and van den Buuse, 2013).

A recent meta-analysis revealed inconsistent findings in eight association studies of rs6265 and alcohol abuse (Haerian, 2013). However, the meta-analysis found that these inconsistencies were largely due to differences between European populations that generally were not associated and Asian populations (particularly Japanese and Taiwanese) that all showed association. It may be that the function of *BDNF* is modified by underlying genetic differences in these two population groups. Taking our study into consideration, it may be that the rs6265 Met allele has a role in alcohol dependence in both European and Asian schizophrenia populations and in Asians without schizophrenia but not in Europeans without schizophrenia due to underlying genetic differences between populations. This is also consistent with several studies reporting different psychiatric phenotype outcomes as well as different allele frequencies for *BDNF* variants due to population differences (Gratacos et al., 2007; Petryshen et al., 2010; Pivac et al., 2009).

One of the clinical features closely linked to alcohol dependence and alcohol use disorders is depression. Depression and poor mental health have been long identified to be closely linked and are risk factors to alcohol abuse (Briere et al., 2013; Davidson, 1995; Weitzman, 2004). *BDNF* variants have also been studied for association with depressive disorders and evidence of association has been found (Lavebratt et al., 2010; Lee et al., 2013; Pei et al., 2012; Ribeiro et al., 2007; Suchanek et al., 2011). Unlike positive reinforcement for alcohol consumption (reward driven drinking), depression may serve as a negative reinforcing factor (drinking behaviour caused by the need to alleviate the effects of depression) for alcohol abuse (Gilpin and Koob, 2008). It is therefore possible that these *BDNF* SNPs play a role in

depression which leads to alcohol dependence although depression was not examined in the present study.

Two studies have demonstrated that mice with low *BDNF* levels consumed more alcohol compared to mice with high *BDNF* levels (Bahi and Dreyer, 2013; Jeanblanc et al., 2009). *BDNF* mainly modulates the activity of the serotonin/dopamine pathway by binding with receptor tyrosine kinase B (TrkB) which induces expression of the dopamine D3 receptor (Jeanblanc et al., 2006). Blockade of the dopamine D3 receptor has been shown to decrease alcohol consumption in rats (Thanos et al., 2005). These data support a key role for *BDNF* as a genetic risk factor in the development of alcohol dependence.

While many studies have proposed that *BDNF* variants are associated with various psychiatric disorders that are generally comprised of multiple and complex clinical symptoms, it may be more profitable to target specific clinical symptoms rather than the primary diagnosis. To further validate our positive association findings on comorbid alcohol dependence, replication studies with larger sample sizes screened for a range of alcohol-related parameters within schizophrenia, along with a non-schizophrenia alcohol dependent sample screened for alcohol-related parameters is required to elucidate the role of rs6265 and rs7103411.

## **5. Conclusion**

We conclude that the *BDNF* gene, particularly the SNPs, rs6265 and rs7103411, play an important role in the development of comorbid alcohol dependence in schizophrenia patients

but it is not clear that they have any role in the development of alcohol dependence in individuals without schizophrenia.

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### **Role of Authors**

Authors SYC, JV and CPM made a substantial contribution to conception and design; the analysis and interpretation of the data and drafted and critically reviewed the manuscript.

RMY, BRL and JPC made a substantial contribution to the conception and design, the acquisition of data and critically reviewed the manuscript.

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### **Conflict of Interest**

All authors declare that they have no conflicts of interest.

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## Tables and Figures

Table 1: Schizophrenia group one *BDNF* genotype association with comorbid alcohol dependence in schizophrenia

SNP	Genotype frequencies			$\chi^2$	<i>P</i>
rs6265	AA	AG	GG*		
Alcohol dependent+	2	18	22		0.152 <sup>a</sup>
Alcohol dependent-	1	33	64	2.853 <sup>b</sup>	0.046 <sup>b</sup>
Odds ratio <sup>c</sup>	5.818	1.587	1.000		
Odds ratio <i>P</i>	0.288	0.460			
rs7103411	CC	CT	TT*		
Alcohol dependent+	2	19	20		0.071 <sup>a</sup>
Alcohol dependent-	1	32	66	4.793 <sup>b</sup>	0.014 <sup>b</sup>
Odds ratio	6.600	1.959	1.000		
Odds ratio <i>P</i>	0.237	0.164			

Alcohol dependent+, schizophrenia patients with alcohol dependence; alcohol dependent-, schizophrenia patients without alcohol dependence

\* Low-risk genotype, due to higher proportion in schizophrenia patients without alcohol dependence

<sup>a</sup> Fisher's exact test

<sup>b</sup> Mantel-Haenszel test for trend in a given direction

<sup>c</sup> Odds ratio calculated with respect to the low-risk genotype

Table 2: Schizophrenia group one *BDNF* allele association with comorbid alcohol dependence in schizophrenia

SNP	Allele frequencies		$\chi^2$	<i>P</i>
rs6265	A	G*	2.435	0.119 <sup>a</sup>
Alcohol dependent+	22	62		
Alcohol dependent-	35	161		
Odds ratio <sup>b</sup>	1.630	1.000		
rs7103411	C	T*	4.051	0.044
Alcohol dependent+	23	59		
Alcohol dependent-	34	164		
Odds ratio	1.880	1.000		

Alcohol dependent+: schizophrenia patients with alcohol dependence; Alcohol dependent-: schizophrenia patients without alcohol dependence.

\* Low-risk allele

<sup>a</sup> Likelihood ratio chi-square *P* value

<sup>b</sup> Odds ratio calculated with respect to the low-risk allele



Table 3: Schizophrenia replication group *BDNF* genotype association with comorbid alcohol dependence in schizophrenia

SNP	Genotype frequencies			$\chi^2$	<i>P</i>
rs6265	AA	AG	GG*		
Alcohol dependent+	7	25	40	8.716 <sup>a</sup>	0.013 <sup>a</sup>
Alcohol dependent-	3	46	113	7.48 <sup>b</sup>	0.003 <sup>b</sup>
Odds ratio <sup>c</sup>	6.592	1.535	1		
Odds ratio <i>P</i>	0.011	0.336			
rs7103411	CC	CT	TT*		
Alcohol dependent+	7	27	38	8.153 <sup>a</sup>	0.017 <sup>a</sup>
Alcohol dependent-	3	53	106	6.332 <sup>b</sup>	0.006 <sup>b</sup>
Odds ratio <sup>c</sup>	6.509	1.421	1		
Odds ratio <i>P</i>	0.011	0.495			

Alcohol dependent+, schizophrenia patients with comorbid alcohol dependence; alcohol dependent-, schizophrenia patients without comorbid alcohol dependence

\* Low-risk genotype, due to higher proportion in schizophrenia patients without alcohol dependence

<sup>a</sup> Likelihood ratio chi-square

<sup>b</sup> Mantel-Haenszel test for trend in a given direction

<sup>c</sup> Odds ratio calculated with respect to the low-risk genotype

Table 4: Schizophrenia replication group *BDNF* allele association with comorbid alcohol dependence in schizophrenia

SNP	Allele frequencies		$\chi^2$	<i>P</i>
rs6265	A	G*	7.424	0.006 <sup>a</sup>
Alcohol dependent+	39	105		
Alcohol dependent-	52	272		
Odds ratio <sup>b</sup>	1.940	1.000		
rs7103411	C	T*	6.029	0.014
Alcohol dependent+	41	103		
Alcohol dependent-	59	265		
Odds ratio	1.790	1.000		

Alcohol dependent+: schizophrenia patients with comorbid alcohol dependence; Alcohol dependent-: schizophrenia patients without comorbid alcohol dependence

\* Low-risk allele

<sup>a</sup> Likelihood ratio chi-square *P*-value

<sup>b</sup> Odds ratio calculated with respect to the low-risk allele

Figure 1: Association of risk taking behaviour with *BDNF*.

Risk taking behaviour score (mean and  $\pm$ SE) with BDNF genotypes for, rs6265 and rs7103411. ANOVA post-hoc multiple comparison was performed for rs6265 and rs7103411 using AA and CC genotypes respectively as comparison.

Footnotes

\*Tukey  $P < 0.05$

\*\*Tukey  $P < 0.01$